

DEVELOPMENT OF OVULE AND TESTA IN RUTACEAE II*: THE UNITEGMIC AND PACHYCHALAZAL SEED OF *GLYCOSMIS* CF. *ARBOREA* (ROXB.) D.C.

F. D. BOESEWINKEL and F. BOUMAN

Hugo de Vries-laboratorium, Universiteit van Amsterdam

SUMMARY

The ovule of *Glycosmis arborea* is anatropous, crassinucellate and unitegmic. This constitutes the first record of unitegmy in Rutaceae. The ovule primordium is tri-zonate. Both subdermal and dermal cells are involved in the initiation of the single integument.

The seed coat formation is quite different from that found in other rutaceous taxa. The post-fertilization development of the ovule is characterized by a pronounced pachychalazy, so that the testa is mainly of chalazal origin. The well-developed vascular system does not extend into the tegumentary part of the relatively undifferentiated testa, only the outer epidermal layer showing wall thickening. Endosperm, nucellus and the inner part of the testa are ultimately almost completely resorbed. As regards its ovule and seed characters, *Glycosmis* can be interpreted as a more advanced genus within the Aurantoideae.

1. INTRODUCTION

According to the résumés of the publications on seed coat anatomy and embryology in the hand-books of NETOLITZKY (1926), CORNER (1976), SCHNARF (1931) and DAVIS (1966), the ovule of the Rutaceae is anatropous, bitegmic and crassinucellate. As in other large and varied families, our knowledge of seed coat structure of the Rutaceae is "tantalizingly inadequate" (CORNER 1976). Of only a small part of the genera (about 150) the seed coat structure has been described. However, from the available data it is clear that Rutaceae show a great diversity in their seed coat anatomy. On the basis of the position and structure of the principal mechanical layers Corner distinguishes five main kinds of seed coat structure in this family, with several minor variations.

His fifth type characterized by an undifferentiated, not or scarcely lignified, testa (s.s.), and an unspecialized tegmen, comprises the genus *Glycosmis* and possibly *Chloroxylon*.

According to Corner's description of *Glycosmis* the ovule is bitegmic. The inner integument becomes obliterated during the later stages of seed coat development. His figures do not show a clear distinction in an outer and an

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inner integument. Also GALLET (1913), DESAI (1962) and NARAYANA (1963), reported that in *Glycosmis* the ovule is bitegmic. *Glycosmis* is the only genus of the Rutaceae the seed coat of which is known to possess an extensive network of vascular bundles (GALLET 1913, CORNER 1976). In this study it will be demonstrated that the ovule and seed anatomy of *Glycosmis arborea* deviates rather markedly from the general rutaceous type by the incidence of unitegmy and pachychalazy.

According to DAVIS (1966) about 5% of the families of angiosperms include taxa with both unitegmic and bitegmic ovules. These families are of special interest for studies aimed at an elucidation of the phylogenetic change-over from bitegmy to unitegmy. This study proves once more that the process of "unitegmisation" is not as unique as was formerly surmised and that it has occurred several times during the evolution of the angiosperms and comes about along different ontogenetic pathways. A detailed ontogenetic and histogenetic study is necessary to acquire a deeper understanding of the evolutionary change from bitegmy to unitegmy.

2. MATERIAL AND METHODS

The material was collected in the Botanical Garden (Hortus Botanicus), University of Amsterdam. It appeared to be impossible to identify the material unequivocally. According to Dr. B. C. Stone (Kuala Lumpur), the taxonomic literature relating to the genus *Glycosmis* is exceedingly confusing. The species in question is commonly cultivated in botanic gardens under the names of *Glycosmis pentaphylla* (Retz.) Corr. or *Glycosmis arborea* (Roxb.) D.C. The species will be referred to here as *G. arborea*. Fixation took place in Craff or Allen-Bouin. Microtome sections were made following standard procedure and stained with saffranin and astra blue. In addition hand sections were made for specific staining with phloroglucinol-hydrochloric acid, Sudan IV, Ruthenium oxychloride and iodine in potassium iodide solution. SEM photographs were made with the aid of a Cambridge Stereoscan mark 2A. The specimens were sputter-coated with gold for about 4 minutes.

3. RESULTS

Glycosmis arborea has a 4-loculed ovary with mostly one ovule or occasionally two superposed pendulous ovules per locule. According to SWINGLE & REECE (1967) the genus *Glycosmis* must be referred to the subfamily Aurantoideae, tribe Clauseneae, subtribe Clauseninae. The fruit is baccate and, since only one ovule develops into a large seed, always 1-seeded.

In contrast to many representatives of the Aurantoideae no "juice sacs" (= groups of endocarp cells) are formed.

Ovule ontogeny

The ovule, like those of all previously studied representatives of the Rutaceae, is trizonate. Its initiation takes place by periclinal divisions in the corpus (L3), which is surrounded by the initially mainly anticlinally dividing layers of the subdermatogen (L2) and dermatogen (L1) – see *fig. 1A, B*. The nucellus is for the most part formed by repeated periclinal divisions of the subdermal layer. Already before the reduction division takes place, the epidermis of the nucellus starts dividing to form a nucellar cap (*fig. 1C, D*). The nucellus attains an appreciable size. The megaspore tetrad is linear. Between the embryo sac and the nucellar cap a distinct parietal tissue is formed (*fig. 1E*). The integument is already initiated in a very young developmental stage by both dermal and subdermal cell divisions. Its primordium is ring-shaped and also clearly developed at the raphe side although here it is mainly of dermal derivation. About four adjacent dermal cells start dividing by forming periclinal and oblique walls (*fig. 1A, B*). Below, and slightly distally with respect to these dermal mitoses, periclinal cell divisions take place in the subdermal layer. The cells formed by these periclinal divisions push the dermal cells upwards, so that a partly dermally initiated integument is formed. The dermal part is more or less adaxially situated with respect to the subdermal portion (see *fig. 1C, D*). In later stages of development the border line between the dermally and the subdermally formed tissue becomes obscure. Already soon after its initiation the integument is about five cell layers thick, but the number of cell layers only increases further in the post-fertilization phase.

The full-grown ovule

The ovule is unitegmic, anatropous, and crassinucellate when fully developed (*fig. 2*). The raphe already contains a differentiated vascular bundle which divides into a number of procambial strands in the chalaza. Raphe, chalaza, and integument contain many cells filled with tannic substances: in the raphe and the chalaza these cells are mainly centred around the vascular system. Starch grains occur throughout the ovule but principally in the embryo sac (*fig. 1F*), in the nucellar cap, in the apical region of the integument, and in the hairs of the obturator. The micropyle does not become completely closed: the remaining small opening is blocked by the nucellus cap (*fig. 1E, figs. 2 and 3*).

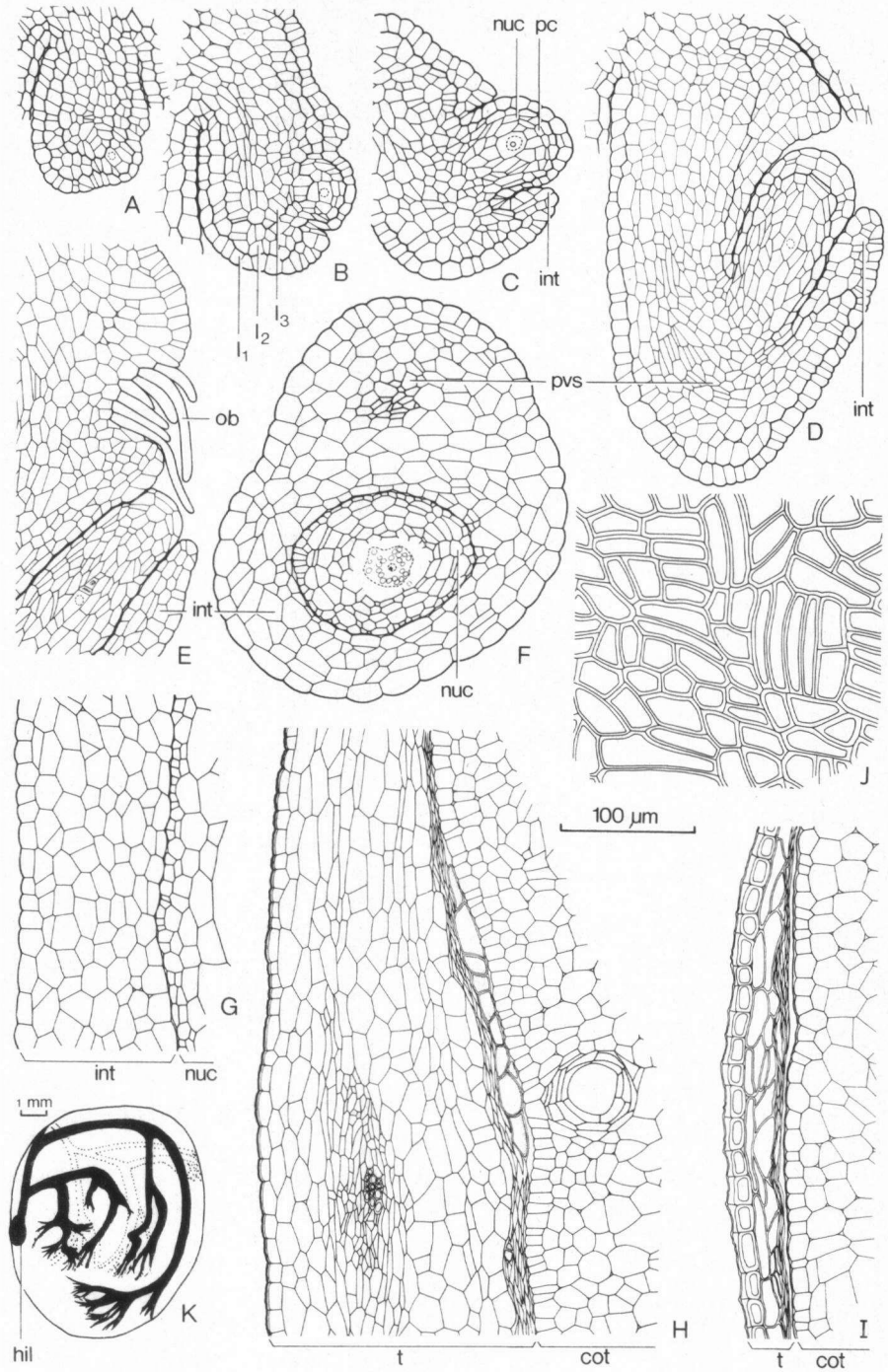
The obturator

Shortly before the ovule is fully developed, an obturator is formed by the stretching of dermal cells which are continuous with the epidermis cells of the stylar canal. It consists exclusively of unicellular hairs growing in the direction of the micropyle (*figs. 1E, 4*). It degenerates during the postfertilization stage.

The development of the seed coat

During the development of the fertilized ovule into a seed the spatial relationships between the various constituting parts change drastically.

The greatest increase in size is caused by a marked proliferation of the



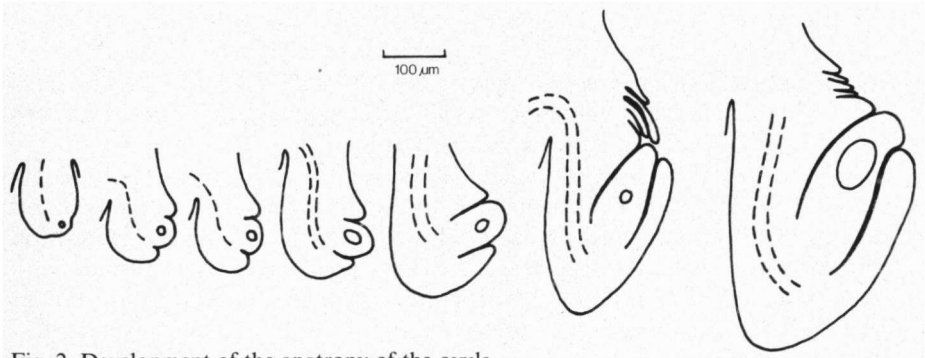


Fig. 2. Development of the anatropy of the ovule.

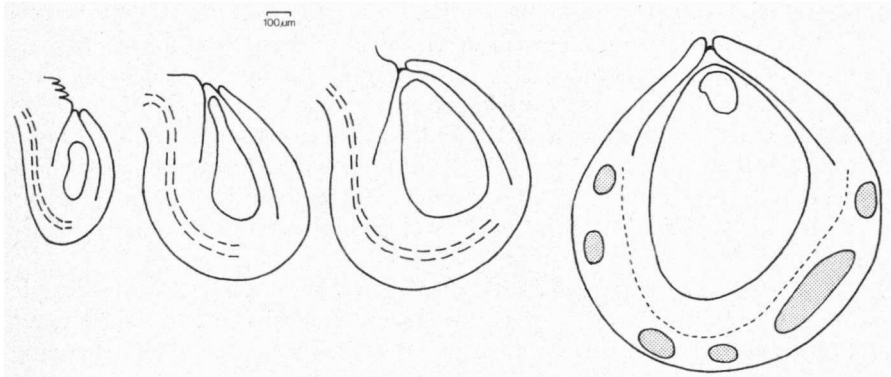


Fig. 3. Early stages of developing seeds, showing massive growth of chalaza (three median and one transmedian sections are shown).

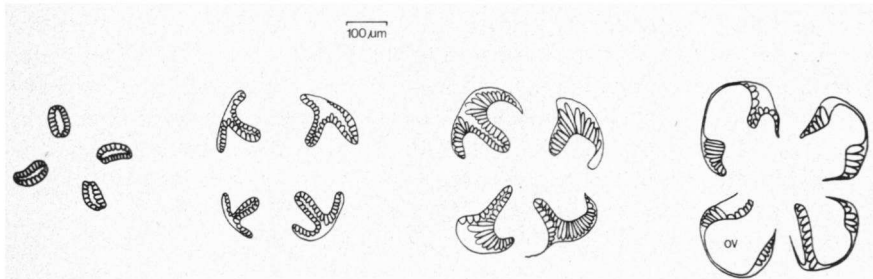


Fig. 4. Styler canals and obturator hairs as seen in cross sections at different levels of ovary.

Fig. 1. Development of ovule and testa of *Glycosmis arborea*.

A, B, C, and D: l.s. of developing ovules; E: part of l.s. of almost full-grown ovule; F: tr.s. of fully developed ovule; G, H, and I: development of seed coat as seen in tr.s. of developing seed. J: fully developed seed coat seen from above; K: ripe seed showing vascular bundles in testa.

L1, L2, L3 = dermal layer, subdermal layer and corpus, respectively; es = embryo sac; nuc = nucellus; pc = parietal cells; end = endosperm; int = integument; t = testa; pvs = provascular strand; ob = obturator; cot = cotyledon.

chalazal tissues. In a fully developed ovule the tegumentary region is about as large as the chalazal part (*fig. 1D, 2*), but during the subsequent development into a seed the latter becomes progressively and relatively larger (*fig. 3*). This chalazal proliferation is mainly the result of the meristematic activity of cells lying within the chalazal network of vascular tissue (*fig. 5E*). At seed maturity the biomass ratio between the tegumentary and the chalazal parts ultimately becomes about 1 to 5. Concurrently with the increase in size of the chalazal portion of the growing seed the embryo sac becomes larger by the resorption of the central chalazal tissue (*fig. 5E*). The funicular vascular strands and those in the adjacent part of the raphe are surrounded by a sheath of tannin cells, and the ramifications of these strands are also provided with such a sheath, so that those parts of the testa which are of chalazal and raphal derivation exhibit a distinct stratigraphy of five layers, *viz.*: (see *fig. 5D, F*) an epidermis (also tannin-containing), an outer subdermal and tanniferous layer of parenchyma, a starch-containing layer with vascular bundles, an inner tanniferous zone particularly well developed at the inside of the vascular bundles (and sometimes continuous with the outer tanniferous layers between these bundles), and an innermost parenchymatic layer without starch grains which is richer in cytoplasm and more or less meristematic during the younger stages (*fig. 5E*). The inner tanniferous layer possibly has the characteristics of a hypostase.

The tegumentary part of the seed coat does not contain vascular strands and is not many-layered. The epidermis has the same structure as in the other part of the seed. The deposition of tannic substances commences mostly in the subdermal layers of cells and in the apical portion of the integument. In later stages tannin is also formed in other cells of the integument.

The nucellus does not form a clearly discernible cuticle and becomes compressed so that during the later stages of seed coat development it is hardly distinguishable from the integument, and ultimately disappears completely.

The endosperm is initially nuclear and of modest dimensions only, and becomes cellular at a much later stage. Conceivably the endosperm formation contributes towards the enlargement of the embryo sac by acting as an unspecialized haustorium. In a young seed with almost fully developed embryo hardly any endosperm remains (*fig. 1H*: stippled cells).

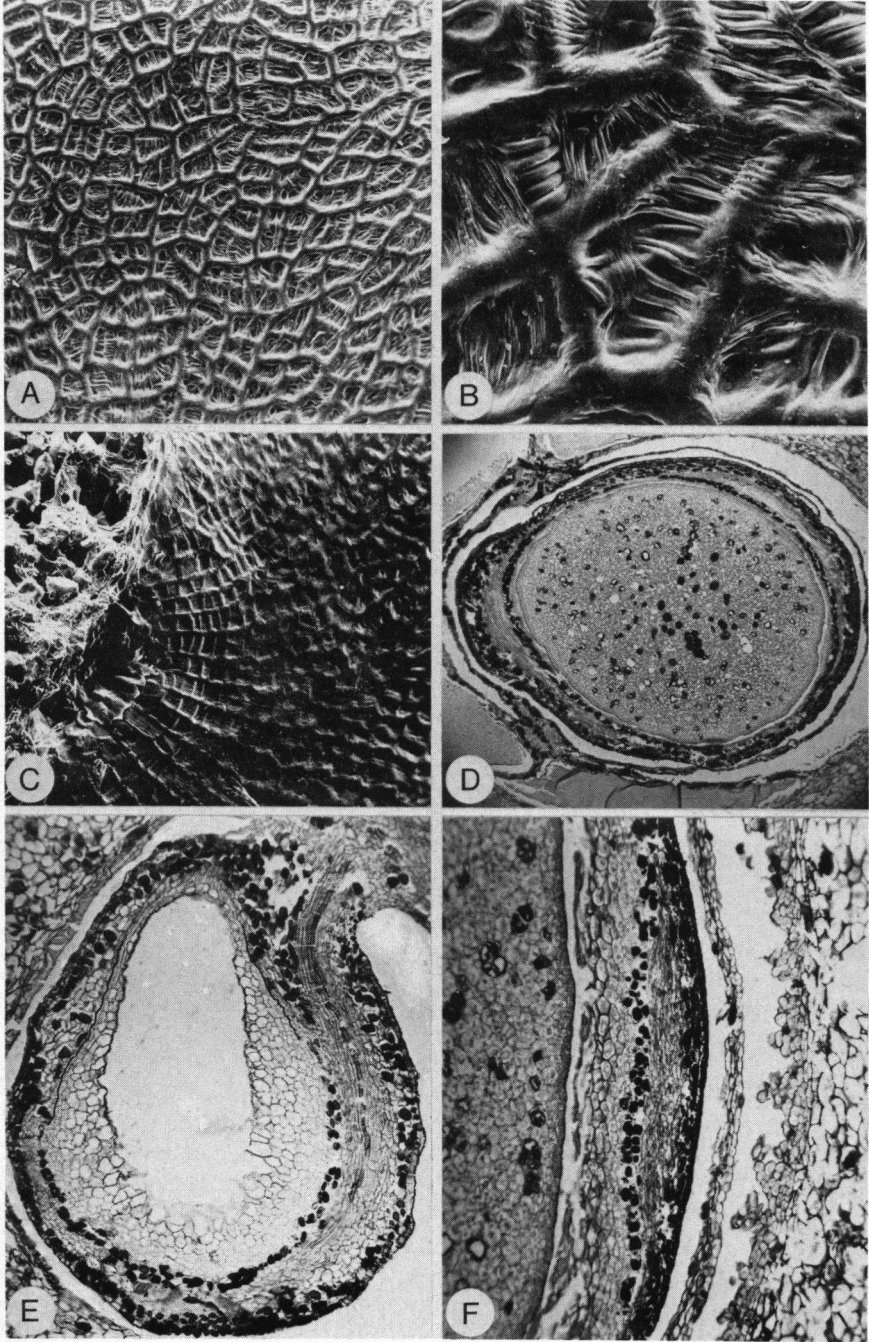
The raphe bundle and its ramifications are amphicribal.

The mature seed

The only well-differentiated cell layer of the seed coat is the epidermis. Its outer walls have a thin cuticle overlying a faintly cutinized cellulosic wall

Fig. 5. A, B: fully developed seed coat, (SEM, magnif. 100 diam. and 900 diam.); C: Micropylar region (SEM, magnif. about 100 diam.); D: l.s. of young seed (magnif. about 25 diam.); E: median l.s. of developing seed shortly after fertilization ($\times 80$); F: Detail of testa shortly before maturity ($\times 80$).

D, E and F are standard photomicrographs.



layer poor in pectic substances. The cell lumen is surrounded by a stratified wall strongly reacting with Ruthenium Red. Not a single element of the testa is lignified (*fig. 1I*). The radial walls are responsible for the reticulate texture as seen in the SEM photographs *5A* and *5B* and *fig. 1J*: the finer lines are wrinkles in the outermost cell wall layer of the testa. The epidermis exhibits a distinct cell pattern in which the most recent divisions are still discernible. Owing to the massive growth of the chalazal region the epidermal cells do not show a clear orientation except in the micropylar part (*fig. 5C*), where they are arranged in short, radiating rows.

When the seed is full-grown the embryo still increases in size to some small extent by the compression and partial resorption of the inner subdermal layers of the seed coat. In the mature seed coat only 2–4 cell layers of the outer tanniferous parenchyma are still intact, which give this coat a papyraceous texture. Along the line of separation of the cotyledons the seed coat is somewhat thicker and its subdermal cells are provided with wall thickenings and pits. The seed coat is also slightly more tumid along the partly compressed vascular bundles. The mature seed does not contain any remains of the nucellus and the endosperm, the embryo filling the whole seed cavity and being closely adpressed to the seed coat.

The embryo has thick, plano-convex cotyledons, a small radicle and a hairy plumule (see also CORNER 1976 I: 235). The cotyledons are rich in starch but do not contain a great deal of fatty substances, their tissues contain scattered groups of tanniferous cells and also schizolysigenous oil glands. The mature seed is subglobose, slightly compressed in a longitudinal direction, and about 7 mm × 8 mm in size.

When fresh the seed is of a dark green colour owing to the presence of chlorophyll in the cotyledons, against which the vascular bundles are conspicuous as a paler venation. The micropyle lies very close to the hilum (*fig. 4C*). The fruit is pale pink when ripe and has a sweet and spicy taste.

Germination of the seeds may already take place immediately after the fruits have been collected.

4. DISCUSSION

Glycosmis cf. *arborea* deviates from all other Rutaceae studied so far by its unitegmatic ovules and the pachychalazal proliferation of the seed. The integument is single in every stage of its development, but its histogenesis is suggestive of the origin of the unitegmatic condition by "integumentary shifting" as described by BOUMAN & CALIS (1977) for certain Ranunculaceae. During the early initial phase already periclinal divisions take place in the dermal layer, comparable with the initiation of an inner integument. Almost simultaneously, and slightly proximally of these divisions in the dermal layer, subdermal divisions occur in the way which is characteristic of the outer integument initiation of the Aurantoideae: these divisions cause an upward and more or less adaxial shift of the dermal derivatives with respect to the subdermal

ones. The integument is also rather thick from the very beginning. Moreover, the contribution of the dermatogen is greater at the raphal side than at the dorsal side of the ovule. A study of other species of *Glycosmis* may yield additional arguments to support the suggested phylogenetic origin of unitegmy in *G. arborea*.

The development from ovule to seed is characterized by the extension of the vascularized chalazal region. CORNER (1976, part I: 5), following Periasamy, calls such seeds in which the chalaza forms a container for the embryo a "pachychalazal" seed. Such seeds he considers to be derived. The vascular bundles run from the hilum and the raphal region through the chalazal part in the direction of the micropyle to form terminal ramifications and anastomoses, which are all approximately equidistant with respect to the micropyle. The small part of the seed coat around the micropyle which is not vascularized is the only one derived from the original integument, so that *Glycosmis* agrees with all other Rutaceae in the absence of tegumentary vascular bundles. The function of the vascular bundles in *Glycosmis* has most probably something to do with the necessity of supplying a sufficient amount of nutrients during the rather rapid development of the seed.

CORNER (1976 I: 50) has surmised that the simple, unitegmic ovules of sympetalous orders are derived from neotenually developed pachychalazal seeds. However, the development of the ovule and seed of *Glycosmis* does not provide any indications of a relation between pachychalazy and unitegmy, because the unitegmic condition originates ontogenetically in a much earlier stage of development than the chalazal proliferation (which only begins after the fertilization has been completed). The single integument and pachychalaza of *Glycosmis* quite clearly point to a phylogenetically more derived condition of this genus with respect to the other representatives of the Rutaceae-Aurantoideae (at least as far as the seed characters are concerned). Some fruit characters are also indicative of an advanced status of the genus *Glycosmis*: the ovarial locules have usually one ovule, and the fruit contains often only a single, large seed, the pachychalazal proliferation permitting the development of a large seed out of a relatively small ovule. According to CORNER (1976) the decrease of the number of seeds per fruit may often result in an increase in size of the remaining seeds (or seed).

SWINGLE & REECE (1967) refer *Glycosmis*, together with *Murraya* and *Clausena* to the (supposedly) primitive tribe Clauseneae, subtribe Clauseninae. Also in the treatment of the family in ENGLER & PRANTL (1931) *Glycosmis* appears "at the beginning" of the Aurantoideae. In view of the above-mentioned derived and unusual features the inclusion of *Glycosmis* in the primitive tribe of the Clauseneae appears to be highly questionable. It is not unlikely that among the genera referred to the primitive tribes there are some, such as *Micromelum* (with a somewhat leathery seed coat) and *Luvunga* (with a veined testa) which are in fact more derived at least in their seed characters. A more detailed analysis of the development of ovule and seed in such Aurantoideae seems promising and is certainly indicated.

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